Translation and Optimization of Supercritical Fluid Extraction Methods to Commercial Instrumentation

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Abstract

Acceptance of supercritical fluid extraction (SFE) is partially impeded by the lack of confidence that analytical SFE methods can be reproduced on varying types of instrumentation. In this study, we have attempted to optimize and translate several SFE methods and techniques developed in our laboratories on noncommercial apparatus onto commercial instrumentation. The test cases involved the separation of incurred organochlorine pesticides from coextracted lipid material, total oil extraction from soybean flakes, and the extraction of various pesticide moieties from wheat. Utilizing four different commercial instruments, we achieved over 90% recovery of the pesticide and lipid moieties from the above sample matrices using only supercritical CO_2 at various extraction pressures. Reproducibility of the extractions on a particular instrument varied depending on the test method and the nuances of the extraction technique. Overall, the agreement between results obtained on each of the above instruments for a particular test was excellent; however, oil extraction results showed a pronounced dependence on extraction pressure.

Introduction

The rapid development of supercritical fluid extraction (SFE) as an analytical technique is well documented in the literature (1,2). However, further growth of the technique is dependent on the availability of instrumentation that can be applied to a wide variety of analytical problems and sample matrices. Many of the reported SFE methods to date have been developed on noncommercial apparatus (3–5) by investigators who have demonstrated that SFE is a highly accurate and reproducible technique. However, such methods may never be adopted for routine use, unless they can be successfully translated onto commercial modules by analysts who may lack considerable expertise in SFE.

In a recently published study (6), Lopez-Avila and coworkers found that results for the SFE of soil samples were identical on four different commercial SFE systems. However, extraction efficiencies were low for some analytes from these matrices due to restrictor plugging and interaction with the sample matrix. It would seem prudent, therefore, to optimize extraction conditions before comparing results on available instrumentation. Recent round-robin studies sponsored by the EPA (7) and NIST-CALS (8) also substantiate the need for more fundamental research on optimizing extraction conditions for the SFE of environmental samples. Such studies should lead to even further improvements in instrumentation for SFE (9), and allow the technique to be integrated into many laboratory operations.

As part of a continuing research program to develop analytical SFE methods and instrumentation for regulatory analysis, we decided to initiate a study to evaluate the adaptability of commercial instrumentation to methods previously developed in our laboratory (10–12). The objectives of our study were as follows: (1) to translate SFE methodology to commercial instrumentation; (2) to test whether SFE-derived results are instrument dependent; and (3) to determine precision levels for specific extractions on various instrument modules. To accomplish the above goals, three generic test methods were selected, representative of different types of analytes, sample matrices, and collection methods. These consisted of the following types of extractions: (1) pesticide extraction from poultry fat with in-situ cleanup; (2) exhaustive delipidation of soybean meal; and (3) pesticide extraction from wheat samples.

Method 1 involves the extraction of incurred pesticide residues from poultry fat in the presence of an alumina sorbent so as to permit cleanup of the target analytes from coextracted lipid matter before electron capture detection–gas chromatographic (GC–ECD) analysis (13). It is particularly sensitive to flow-rate variance through the extraction cell, which controls the breakthrough volume of the pesticide fraction and retardation of the fat fraction on the alumina cleanup column. Hence, both target-analyte recovery and effectiveness of cleanup can be adversely affected if the fluid flow rate is erratic.

Method 2 is a potential substitute for Soxhlet extraction of oils from seed matrices. Exhaustive lipid extraction can best be conducted at high pressures and temperatures (14), and rapid extractions can be realized if the flow rate is sufficient. In such a method, pressure limitations in the extraction apparatus can severely extend the time of extraction, as well as limit the recovery of the total oil content of the sample.

Methods 3 is also an SFE of pesticides, except in this case, the pesticides are directly extracted from a spiked-wheat matrix.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.
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Two collection methods were employed in this protocol: isolation of the extracted analytes in a solvent-laden vial and on a sorbent-filled cartridge trap. This not only permitted a comparison between the performance of the various instruments, but allowed the detection of any bias in the analytical results due to the collection technique.

Experimental

Instrumentation. The following four instruments were used in this study: an Isco SFX 2-10 extractor, a Hewlett Packard 7680A SFE system, a Suprex Prepmaster unit, and a Lee Scientific Model 703 extractor. The extractions designated NCAUR were performed on equipment constructed in our laboratory and described in the literature (15). SFE-grade CO_2 with helium headspace was used in all extractions performed on the Suprex, Lee Scientific, and Isco extractors. Extractions conducted on the Hewlett Packard 7680A utilized neat SFE-grade CO_2.

Extraction methodologies

Pesticides in poultry fat

SFE of three pesticide residues in poultry fat from coextracted lipid matter over an alumina sorbent was performed on the four instruments. On the Hewlett Packard Model 7680A, extractions were conducted using a 7-mL extraction thimble loaded with 1.8 g alumina and 0.2 g chicken fat. Extractions were performed at 3,100 psi and 50°C, using a CO_2 flow rate of 3 mL/min for 18 min, with subsequent collection of the pesticides on a trap filled with stainless-steel balls. The collection trap was then rinsed with hexane at 1 mL/min for 6 min, and the eluent was collected in vials for subsequent analysis. The pesticides were found to be completely desorbed into the first collection vial. Less than 0.02% of the available fat was unretained by the alumina column.

On the Suprex Prepmaster, pesticide extractions from poultry fat were accomplished using 5 mL extraction vessels filled with 1.8 g alumina and 0.2 g fat. After sealing, the extraction vessel was placed vertically in the Prepmaster oven, and CO_2 flow commenced from the bottom to the top of the extractor vessel. Extraction pressure was 3,800 psi and the extraction temperature was held at 50°C. The extractions were conducted for 35 min at a liquid CO_2 flow rate of approximately 2 mL/min. Collection was accomplished in a vial containing 8 mL of hexane.

Five extractions were performed with the Isco SFX 2-10 using a 2.5-mL extraction cell loaded with 1.85 g alumina and 0.2 g chicken fat. Extractions were conducted at 250 atm (3,675 psi) and 50°C. Approximately 50 mL of liquid CO_2 was used in performing each extraction. Analyte collection was accomplished through a fused-silica back-pressure restrictor (35 cm × 50-µm i.d.) into a vial containing 10 mL hexane. The alumina was effective in retaining 99.42 wt.% of the poultry fat.

Poultry fat and pesticide extractions, run on the Lee/Dionex 703 unit, consisted of eight extractions conducted simultaneously, as opposed to a series of single extractions conducted on the other three units. The extraction pressure and temperature were 250 atm and 50°C, respectively, with the restrictor temperature held at 100°C. The collection vials containing 5 mL of hexane were held at 0°C. The amounts of poultry fat and alumina charge in the 3.5 mL extraction cells were the same as described for the other extractor modules. The total volume of CO_2 passed through each individual extraction cell was 10–15 L on an expanded volume basis. Fat retention on the alumina sorbent was 99.8 wt.%.

The alumina used in all the above extractions was neutral, Brockman Activity 1, 80-200 mesh (Fisher Scientific), which was heated for 4 h at 800°C, then cooled to room temperature before the addition of 5% water to regulate its final activity. Pesticide extracts in hexane were evaporated to 0.5 mL; and 0.5 mL of an internal standard, consisting of 100 pg/µL aldrin in isooctane, was added prior to GC/ECD analysis.

Oil in soybean flakes

As noted before, the optimal conditions for the extraction of soybean oil from flaked meal are 10,000–12,000 psi and 80°C. However, these conditions could only be obtained on one of the SFE units, the Lee/Dionex 703 extractor. In some cases, for the other instrumentation used in this study, options exist that allow extractions to be performed at the elevated pressure range quoted above. Unfortunately, these options were not available to us at the time this research was initiated, resulting in certain oil extractions reported in this study being conducted under less-than-optimal conditions.

For the Lee/Dionex 703 system, three sets of parallel extractions were performed under the following conditions: an extraction pressure of 645 atm, oven temperature of 80°C, a restrictor temperature of 150°C, and restrictors calibrated to 500 mL/min at 340 atm. Collection vials containing glass wool inserted into a tube inside the vial were used to condense the oil after depressurization. One experimental run was conducted for 30 min using a sample size of 1.5 g in a 3.5-mL cell, while two other runs utilized 4-g samples in a 10.0-mL cell using a 60-min extraction.

Six soybean oil extractions were performed on the Isco SFX 2-10 system, using extraction conditions of 7,500 psi and 80°C. A total volume of 150 mL liquid CO_2 was used in each run. A 35 cm × 50-µm i.d. fused silica backpressure restrictor, heated to 65°C, was used to maintain CO_2 flow and pressure. Soybean flake samples, ranging from 3.3–4.1 g by weight, were extracted in a 10-mL cell. An empty test tube was used to collect the oil.

Soyflakes were extracted on the Prepmaster system using 2-g samples in a 5-mL vessel. The extraction parameters were 7,350 psi and 80°C, and the extracted oil was collected in either hexane or an empty vial. The restrictor temperature was kept at 80°C. With the Hewlett Packard 7680A, soybean flakes were extracted using a 7-mL thimble containing approximately 3 g flakes. Extractions were performed at 5,300 psi and 80°C, close to the upper-pressure limit of the unit. Several trapping options were used, including a trap filled with stainless-steel balls followed by a hexane wash, as well as direct trapping of the oil by precipitation into a flask.

Soybean flakes were also extracted on noncommercial equipment constructed in our laboratory to provide a basis for comparison with the results obtained from the commercial modules and Soxhlet extractions. Four runs were conducted at 7500 psi, 80°C, using a total of 50 L CO_2/run, as measured on an expanded volume basis. The CO_2 flow rate was approximately 2 mL/min and the neat oil was collected in 250-mL round-bottom flasks. Approximately 4.0 g soybean flakes were placed in a 6-in. long × 1-in. i.d. cell for each extraction. An additional four extractions were also performed under similar conditions, with the exception that the extraction pressure was 10,000 psi and the total volume of CO_2 used per run was 300 L at a flow rate of 5 L/min.

In the above experiments, the precipitated oil was rinsed down into the collection flask with a minimal amount of hexane. The oil was than rotary evaporated to remove the hexane until a constant weight was achieved. It should be noted that in all of the soyflake

extractions, only gravimetric determination of the oil was used as a criterion for determining the percent oil in the flake samples. Both the rinse and collection solvents were removed by evaporation, when necessary, in the above experiments. The collected oil was also heated after extraction to remove traces of coextracted moisture and drive off imbibed CO_2 dissolved or entrapped in the viscous oil.

Pesticides in wheat

The optimum extraction conditions for the removal of four pesticides from wheat were established in a previous study (16). The wheat samples were spiked at a 5-ppm level using pesticides that could be detected via GC/ECD methods. The selection of a collection sorbent for the trap was based on integrating SFE with an established traditional protocol (17) that uses Florisil.

Wheat samples were extracted on the Isco SFX 2-10 system at 5000 psi, 60°C, using 50 mL of liquefied CO_2. Approximately 5 g wheat was placed in a 10-mL cell for each of the extractions. A fused silica restrictor, 35 cm × 50-µm i.d., was used as a backpressure restrictor for the extractions, and the analytes were collected in a vial containing 15 mL ethyl acetate. Four extractions utilized a sorbent trap for pesticide collection. Extraction conditions were identical to those employed for analyte collection into the ethyl acetate; however, 1.25 g Florisil packed in a stainless-steel trap was used to collect the analytes after CO_2 decompression. After completion of each extraction, the fused-silica restrictor was disconnected, and a piece of stainless-steel HPLC tubing was connected between the SFX 2-10 cell outlet and the sorbent trap. The 10-mL extraction cell was replaced with an empty 2.5-mL extraction cell, and the second pump in the SFX 2-10 system was utilized to deliver ethyl acetate through the trap to elute the pesticides.

The latter procedure described above for the Isco SFX 2-10 system could be done automatically on the HP 7680A unit. Here the extractions were made at 5,000 psi and 60°C with 50 mL CO_2, using a 7-mL extraction thimble containing 2-g samples. The Florisil trap was held at 40°C. Pesticides collected on the Florisil trap were eluted with acetone.

Wheat extractions conducted on the Prepmaster utilized 2.5 g sample in a 5-mL extraction cell. Extraction pressure and temperature were identical to those used on the Hewlett Packard and Isco extractors. Pesticides were collected directly into either 8 mL acetone, or alternatively onto a Florisil trap, which was subsequently rinsed with acetone to remove the pesticides. Triplicate extractions were performed using each mode of collection.

The pesticide extractions from wheat run on the Lee/Dionex 703 consisted of four parallel, simultaneous extractions, each run in 10 mL cells loaded with approximately 5.0 g sample. Extractions were performed at 340 atm (5,000 psi) and 60°C. Restrictor and vial temperatures were 150° and 2°C, respectively. For all the extractions, 10 mL of ethyl acetate was utilized as the collection solvent. One set of extractions utilizing restrictors calibrated to deliver 500 mL/min at 340 atm and 200°C of expanded CO_2 flow, was run for 60 min. Two additional sets of samples each were run using 250 mL/min flow restrictors (calibrated at 350 atm and 200°C) for 2 h each.

Collection and eluent solvents containing the pesticide extracts were all diluted to 100 mL, and a 1.5-mL aliquot was taken and transferred to an autosampler vial for GC/ECD analysis. Quantitation was performed by using an external standard.

Chromatographic analysis

Pesticide recoveries were determined using GC/ECD analysis on a Hewlett Packard 5890A gas chromatograph equipped with a 30 m × 0.32-mm i.d. DB-5 column. Injector and detector temperatures were 220° and 350°C, respectively. Programmed temperature runs consisted of an isothermal hold for 1 min at 100°C, followed by a 10°/min ramp to 190°C, and then a 3°/min ramp to 250°C, with a final isothermal hold at 250°C for 10 min. A 2-µL injection volume was used in a splitless injection mode. Peak quantitation was performed on a Hewlett Packard 3396A integrator.

Results and Discussion

Table I contains the results for the SFE of incurred pesticide residues from poultry fat using the described in situ cleanup method under supercritical fluid conditions. With the exception of the anomalously low value for heptachlor epoxide when extracting chicken #319 on the Lee 703 unit, the mean results for each of the pesticides, from instrument to instrument, are statistically equivalent within the range of their standard deviations. Peritoneal fat extracts from two different birds are given due to an insufficient quantity of tissue sample for running extractions on all four commercial units. An independent study (18) has shown that the concentration of these three incurred residues in peritoneal fat is relatively constant from one chicken to another.

The reproducibility of the extractions determined on one instrument generally gave relative standard deviations (RSD) under 10%. The higher RSD values recorded on the Lee 703 unit represent the variability between eight identical samples run in parallel simultaneously on this instrument. Relative standard deviations for the pesticides extracted on the other instruments represent the variability in results between consecutive extractions run on each specific instrument. It is interesting to note that simultaneous extraction and cleanup of eight samples using an established regulatory protocol (19) yielded an RSD of 7–7.5%. Therefore, the RSD for the multisample results determined on the Model 703 are only slightly higher than those using conventional methodology.

The results for oil extraction from soybean flakes by SFE are summarized in Table II. For purposes of comparison, Soxhlet-derived values on the same substrate, using slightly different extraction solvents, are also listed in Table II from two laboratories, NCAUR and the Total Diet Research Center of the U.S. Food and Drug Administration (TDRC-FDA). It should be appreciated that the fat or oil content of a seed will depend on the chosen extrac-

Table I. Results from SPE and Cleanup of Incurred Pesticide Residues in Poultry Fat

Extractor	Heptachlor epoxide*	Dieldrin*	Endrin*
Lee SFE-703 (#319)	0.64 (10.4%)	2.7 (11.7%)	2.1 (14.2%)
(#388)	0.79 (3.71%)	2.6 (12.7%)	2.1 (9.87%)
HP 7680A (#319)	0.795 (0.91%)	2.8 (10.9%)	2.4 (6.31%)
(#388)	0.78 (3.87%)	2.9 (9.08%)	2.1 (10.2%)
Isco SFX 2-10 (#319)	0.80 (3.71%)	2.9 (6.48%)	2.3 (5.42%)
Suprex Prepmaster (#388)	0.83 (2.42%)	2.8 (2.11%)	2.3 (5.26%)

* In ppm of poultry fat
(%) = % RSD
(#) = Poultry Sample No.

tion solvent (20). In addition, weight percent oil content was determined on noncommercial extraction equipment assembled at NCAUR using two different extraction pressures. The two weight percent oil values derived from the Soxhlet method at NCAUR represent determinations made four months apart, the approximate time span of the total study.

The agreement between the oil-extraction results obtained on the NCAUR extractors and the Soxhlet-derived values are within 0.3 wt.%. The results obtained on the Lee 703 unit are also in agreement with the above-mentioned results. The results obtained on the other three commercial units are, on average, only 90% of those obtained by using either the noncommercial apparatus, the Soxhlet method, or the Model 703. This result is partly due to the extraction-pressure limitations of these other commercial modules, which makes total oil extraction difficult to achieve in the same time frame as higher-pressure supercritical extractions or organic-solvent-based methods. However, RSDs for the oil extractions run on each individual instrument are certainly acceptable, as well as comparable to those reported for Soxhlet methodology (21).

Another interesting result from the soyflake extractions worth noting is reported in Table III. Here, the percent weight loss for the flakes in the extraction vessel is recorded, along with the weight percent oil, as determined from the actual oil collected in the receiver vessel, for the various extraction apparatus tabulated. In all cases, the percent weight loss for the flakes in the extraction vessel exceeds the actual weight percent of oil collected; the latter, however, being in much better agreement with the non-SFE assays for the oil content in the flakes. This is due to the fact that supercritical CO_2 will dissolve approximately 1 mole % (0.4 wt.%) of water under the extraction conditions used in this study (22). Therefore, it would be highly risky to determine the fat or oil content of soybean flakes (approx. 11 wt.% water), or any other high-water-containing matrix, based on its weight loss in the extraction vessel before and after SFE.

It was also observed in the oil extraction studies that the collected oil samples would tend to lose weight after SFE. In one NCAUR-based SFE, this weight loss was observed to take place over 24 h. This phenomena is due to weight loss associated with the slow evolution of imbibed CO_2 from the viscous oil at room temperature. The solubility of CO_2 in triglyceride-based oils at elevated pressures can range from 15–30 wt.%, depending on the system pressure (23). Therefore, it is not unreasonable to expect that all of the CO_2 will evolve from the oil after it has precipitated from the decompressed CO_2. Heating the oil, along with either gas sparging or rotary evaporation, proved effective at quickly reducing the dissolved gas content of the oil.

Table IV shows the results obtained from each instrument for the pesticide extractions from wheat. Here, the results are reported in percent recoveries and respective standard deviation relative to the spiking standard. Also included in Table IV are recoveries obtained on a noncommercial apparatus constructed at NCAUR. These results are reported in an independent study (16). The recovery results for the listed pesticides on the four commercial instruments exceed 90%, and surpass 95% when the results obtained on the Lee 703 with the 500 mL/min restrictors (first data set) are eliminated.

In the two cases where a comparison can be made, the recoveries for the four pesticides, using either a sorbent trap or solvent in a vial for analyte collection, are equivalent. Both trapping techniques yielded 90% or greater for all of the pesticides. Recoveries on the NCAUR-built apparatus, which employed a sorbent trap to collect the extracted analytes, were also high.

The overall results of this study suggest that the selected SFE methods can be successfully translated onto commercially available SFE instrumentation. All of the instruments gave excellent pesticide recoveries, and the reproducibility of the extractions were acceptable. Extractions of oils and fat are best conducted on instrumentation that permits SFE to be performed at extraction pressures approaching 700 atm. Specific commercial instruments, although yielding equivalent analytical results, may be better suited to specific methods, particularly with respect to automating a procedure.

Table II. Results for SBO Extraction from Flakes

Extractor	Wt. % oil	RSD
NCAUR (7,500 psi)	20	6.85%
NCAUR (10,000 psi)	20.6	0.98%
Lee SFE-703	19.3	1.59%
	20.3	0.98%
	20.0	0.80%
Suprex Prepmaster	18.1	0.71%
Isco SFX 2-10	18.2	0.57%
HP 7680A	18.4	2.24%
NCAUR-Anal. Lab (Soxhlet)	20.7	1.44%
	20.5	0.75%
TDRC-FDA (Soxhlet)	20.1	–

Table III. Flake Weight Loss vs. Weight of Oil Collected

Extractor	Flake weight loss	Oil weight
NCAUR (7500 psi)	22 ± 1*	20 ± 1*
NCAUR (10,000 psi)	24.6 ± 0.4	20.6 ± 0.2
Isco SFX 2-10	30.0 ± 1.0	18.2 ± 0.1
HP 7680A	29 ± 1	18.4 ± 0.4
Suprex Prepmaster	28 ± 2	18.1 ± 0.1
Lee SFE-703	29.1 ± 0.4	19.3 ± 0.3
	28.1 ± 0.2	20.3 ± 0.2
	28.2 ± 0.2	20.0 ± 0.2

*Weight %

Table IV. Pesticide Recoveries from Wheat

	% Recovery			
Extractor	Pirimiphos-CH_3	Malathion	Chloryrifos	Dieldrin
NCAUR (t)	101	110	103	91
HP 7680A (t)	100.6 ± 0.2	97 ± 3	95.6 ± 0.5	102 ± 2
Suprex Prep- (s)	98 ± 1	97 ± 10	100 ± 2	99 ± 1
master	98.4 ± 0.6	94 ± 3	98.6 ± 0.6	92 ± 3
Isco SFX 2-10 (s)	100 ± 10	99 ± 8	97 ± 9	96 ± 11
(t)	95 ± 4	95 ± 6	93 ± 3	92 ± 4
Lee SFE-703 (s)	91 ± 9	85 ± 8	86 ± 6	90 ± 9
(s)	96 ± 4	98 ± 2	98 ± 3	103 ± 2
(s)	102 ± 4	98.6 ± 0.6	102 ± 5	203 ± 4

(s) = Solvent
(t) = Sorbent

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